REMARKS

Reconsideration and allowance are respectfully requested.

Claims 31-41 are currently pending, with claim 31 being the sole independent claim.

Claims 31 and 32 have been amended. Support for these amendments can be found in the specification at least at page 6, lines 15-21 and page 1, line 34 to page 2, line 5. No new matter is believed to be added.

Turning now to the Office Action mailed May 14, 2002:

The cross-reference to related applications in the specification has been updated, as suggested.

A clean copy of the previously filed Form PTO-1449 is attached hereto, as requested.

Regarding the Section 112, 1st paragraph rejection, Applicant has amended claim 31 to recite "calcium dependent protein kinase activity", instead of "calcium dependent phosphorylase kinase activity". Withdrawal of the Section 112, 1st paragraph rejection is requested.

Regarding the Section 102(b) rejection, Applicant has amended claim 31 (and 32) to recite the Clustal alignment method "with multiple alignment default parameters of GAP PENALTY=10 and GAP LENGTH PENALTY=10 and pairwise alignment default parameters of KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5". In Table 4 at page 19 in the instant specification, the percent sequence identity of SEQ ID NO:4 to the sequence of Lu et al. (NCBI GI No. 1839597) is given as 87%, according to the Clustal method of alignment, using these default parameters. Withdrawal of the Section 102(b) rejection is requested.

Applicants believe the foregoing is responsive to each of the points raised in the Office Action and request withdrawal of all of the rejections and objections contained therein.

Finally, Applicants voluntarily have amended the specification to correct clerical errors and to remove hyperlinks. No new matter is believed to be added. Furthermore, Applicants have filed simultaneously herewith a 37 C.F.R. 1.825(a) Amendment to Sequence Listing and a Transmittal of Formal Drawings.

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Favorable consideration and passage to issue are solicited. Please charge any requisite fees or credit any overpayment to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Applicants' undersigned may be reached at the below-listed numbers.

Respectfully submitted,

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Dated: 14 November 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes, deleted material is shown within brackets, and inserted material is shown underlined.

IN THE SPECIFICATION:

Paragraph at page 1, lines 3-4:

This application claims the benefit of U.S. Provisional Application No. 60/092,438, filed July 10, 1998, now abandoned, and U.S. Application No. 09/347,801, filed July 2, 1999, and issued on July 17, 2001 as U.S. Patent 6,262,345.

Paragraph beginning at page 1, line 34 to page 2, line 2:

The instant invention relates to isolated nucleic acid fragments encoding protein kinase enzymes. Specifically, this invention concerns an isolated nucleic acid fragment encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3 and an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding calcium dependent phosphorylase kinase or glycogen synthase kinase-3.

Paragraph at page 2, lines 3-5:

An additional embodiment of the instant invention pertains to a polypeptide encoding all or a substantial portion of a protein kinase selected from the group consisting of calcium dependent phosphorylase kinase and glycogen synthase kinase-3.

Paragraph at page 2, lines 6-12:

In another embodiment, the instant invention relates to a chimeric gene encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in production of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

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Paragraph at page 2, lines 13-20:

In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3, operably linked to suitable regulatory sequences. Expression of the chimeric gene results in production of altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

Paragraph at page 2, lines 21-28:

An additional embodiment of the instant invention concerns a method of altering the level of expression of a calcium dependent phosphorylase kinase or glycogen synthase kinase-3 in a transformed host cell comprising: a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of calcium dependent phosphorylase kinase or glycogen synthase kinase-3 in the transformed host cell.

Paragraph at page 2, lines 29-31:

An additional embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or a substantial portion of an amino acid sequence encoding a calcium dependent phosphorylase kinase or glycogen synthase kinase-3.

Paragraph beginning at page 10, line 35 to page 11, line 9:

For example, genes encoding other calcium dependent phosphorylase kinase or glycogen synthase kinase-3 enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used

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directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

Paragraph at page 19, line 13-24:

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS. 5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a calcium dependent phosphorylase kinase. These sequences represent two new corn and rice sequences encoding a calcium dependent phosphorylase kinase and the first soybean and wheat sequences encoding a calcium dependent phosphorylase kinase.

Paragraph beginning at page 6, lines 22 to page 7, line 6:

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410[; see also www.ncbi.nlm.nih.gov/BLAST/]). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ*

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hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

Paragraph at page 18, line 5-21:

cDNA clones encoding protein kinase enzymes were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol. 215*:403-410[; see also www.ncbi.nlm.nih.gov/BLAST/]) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nature Genetics 3*:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

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IN THE CLAIMS:

31. (amended) An isolated polynucleotide comprising:

(a) a nucleotide sequence encoding a polypeptide having calcium dependent protein [phosphorylase] kinase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 90% identity based on the Clustal alignment method with multiple alignment default parameters of GAP PENALTY=10 and GAP LENGTH PENALTY=10 and pairwise alignment default parameters of KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5, or

(b) the complement of the nucleotide sequence.

32. (amended) The polynucleotide of Claim 31, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 95% identity based on the Clustal alignment method with the multiple alignment default parameters and the pairwise alignment default parameters.

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